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BRIEF COMMUNICATION

Spatial Learning Deficit and Reduced Hippocampal ChAT Activity in Rats After an ICV Injection of Streptozotocin

ARJAN BLOKLAND[1] AND JELLEMER JOLLES

Department of Neuropsychology and Psychobiology, University of Limburg, P.O. Box 616, NL-6200 MD Maastricht, The Netherlands

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BLOKLAND, A. AND J. JOLLES. *Spatial learning deficit and reduced hippocampal ChAT activity in rats after an ICV injection of streptozotocin.* PHARMACOL BIOCHEM BEHAV **44**(2) 491–494, 1993.—ICV injections of streptozotocin (STREP) lower the glucose utilization of the brain and affect the cholinergic system. The present study was designed to evaluate whether STREP-treated rats have an impaired spatial discrimination performance in the Morris spatial navigation task. Performance in this task is sensitive to treatment with cholinergic antagonists. In contrast to young rats, middle-aged STREP-treated rats tended to have an impaired spatial discrimination performance in the Morris task at the end of training. In middle-aged STREP-treated rats, but not in control rats, spatial discrimination performance was associated with hippocampal choline acetyltransferase (ChAT) activity. The correlation between spatial discrimination performance in the Morris task and the decrease in hippocampal ChAT activity resembles the relation between cognitive and biochemical changes observed in Alzheimer's disease. Our findings suggest that STREP treatment of middle-aged rats may provide a relevant model for dementia.

Brain energy metabolism | Streptozotocin | Spatial learning | Alzheimer's disease
Choline acetyltransferase | Animal model

RECENTLY, Hoyer and coworkers (10,12,13) introduced a model in which brain glucose metabolism is decreased by an ICV injection of streptozotocin (STREP). An ICV injection of STREP impairs the brain insulin–insulin receptor system and consequently leads to a decrease in brain glucose utilization (12). It has been reported that a reduced brain glucose utilization is related to the age-related decline in cognitive function (7,19). A more dramatic decrease in glucose utilization has been observed in dementia (4), suggesting that the severity of cognitive dysfunction is related to the level of brain glucose metabolism. So far, the effects of an ICV STREP-injection on cognition have only been evaluated in inhibitory avoidance learning, where STREP-treated rats show an impaired performance, possibly as a result of a cholinergic dysfunction (10). This explanation is supported by the finding that the level of choline acetyltransferase (ChAT) in the brain is reduced in STREP-treated rats [see (12)].

Spatial discrimination learning has consistently been found to decline with aging (2,16) and be impaired after administration of cholinergic antagonists (9,17). It can thus be hypothesized that ICV STREP-treated rats will also show a deficit in spatial discrimination learning. It has recently been reported that spatial discrimination learning is impaired in patients with Alzheimer's disease, which suggests that the cognitive deficits displayed by Alzheimer's patients might be an expression of an underlying abnormality in the hippocampus and parietal cortex (1). Further, the decline in cognitive functions in Alzheimer's disease is related to a decrease in cholinergic markers (8,15). Thus, evaluation of the effects of STREP treatment on spatial discrimination learning and cholinergic activity could reveal whether the STREP-treated rat can be regarded as an animal model of dementia.

The aim of our study was to evaluate the effects of STREP on spatial discrimination learning in the Morris task. We also subjected rats to an inhibitory avoidance task 1 week after training in the Morris task to evaluate whether we could reproduce the findings of Mayer et al. (10). The behavioral data were correlated with hippocampal ChAT activity.

[1] To whom requests for reprints should be addressed.

EXPERIMENT 1

METHOD

Animals and Surgery

We used 15 4-month-old male Lewis rats, randomly assigned to the control group (CON; $n = 7$) or the STREP group ($n = 8$). All rats were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C). They were kept under a 12 L : 12 D cycle (lights on from 0900–2100 h).

One week before behavioral testing, rats were anesthetized with pentobarbital (60 mg/kg, IP) and placed in a stereotaxic frame. The STREP group was given a bilateral ICV injection of STREP (1.5 mg/kg body weight dissolved in 4 μl saline, 2 μl/injection site). The stereotaxic coordinates were: −0.8 mm anterior, 1.5 mm lateral, and −3.8 ventral from bregma (14). The CON group underwent the same surgical procedures but saline was injected instead of STREP.

Behavioral Procedures

Ten days after surgery, rats were trained on the standard Morris spatial navigation task (11) in a black water tank (diameter 1.22 m). Rats were started from four different, randomly chosen, start positions and trained to find an invisible platform (diameter 11 cm) at a fixed position in the water tank, 1 cm below the surface of the water. The temperature of the water was 20–22°C. A trial lasted until a rat had found the platform or until 60 s had elapsed. If a rat did not find the platform within 60 s, it was placed on the platform for 3 s and then removed from the water tank. On the first day, rats were given four trials. They were given eight trials on the next day and 4 trials on the third day.

Four days after acquisition of the Morris task, rats were subjected to the inhibitory avoidance task. The inhibitory avoidance apparatus consisted of a light and a dark compartment, each measuring 40 × 25 × 40 cm. The light compartment was illuminated by a light bulb (60 W) mounted above (40 cm) the compartment. The floor consisted of a metal grid connected to a shock scrambler. The two compartments were separated by a guillotine door that could be raised 10 cm. On day 1, rats were placed in the light compartment and the latency to enter the dark compartment with four paws was measured. The guillotine door was lowered and a scrambled footshock (0.5 s, 1 mA) was delivered. Twenty-four hours later, a retention trial was given. The latency to enter the dark compartment with four paws was measured.

Statistical Analysis

The step-through latency in the 24-retention session minus the step-through latency during the first trial was taken as the parameter for inhibitory avoidance performance. Treatment effects were evaluated using a *t*-test. For analysis of the Morris task data, the mean escape latency was calculated per block of four trials. Treatment effects were evaluated in a two-factorial (treatment and trial block) analysis of variance (ANOVA) with repeated measures over trial blocks.

RESULTS AND CONCLUSIONS

STREP treatment did not affect the step-through latency during the first trial [step-through latency in seconds (SEM): CON, 9.6 (2.67); STREP, 12.9 (3.67); $t(13) = 1.14$, $p > 0.10$]. Corroborating the findings of Mayer et al. (10), we found that inhibitory avoidance performance was impaired in STREP-treated rats [difference score in seconds (SEM): CON, 234.0 (35.65), STREP, 113.3 (39.30); $t(13) = 2.28$, $p < 0.05$].

In the Morris task, the escape latency of the rats decreased during training, $F(3, 39) = 39.27$, $p < 0.01$ (see Fig. 1), and the escape latency of the STREP group was higher during the acquisition of the task [general mean, $F(1, 13) = 8.54$, $p < 0.05$]. During the last trial block, the performance of CON and STREP-treated rats did not differ, $t(13) = 0.67$, $p < 0.10$. Treatment with cholinergic antagonists results in an impaired spatial discrimination performance at the end of training (17). The STREP-induced performance deficit was therefore not comparable to the pattern normally observed in rats treated with cholinergic antagonists. Because no behavioral deficit was found in STREP-treated rats at the end of training, hippocampal ChAT activity was not determined. We assumed that young rats are able to reverse/compensate for the effects of STREP treatment.

EXPERIMENT 2

METHOD

Animals and Surgery

We used 16 17-month-old male Lewis rats, randomly assigned to the control group (CON; $n = 8$) or the STREP group ($n = 8$). Housing conditions and surgical procedures were identical to those of Experiment 1. One STREP-treated rat died during training for unknown reasons.

Behavioral Procedures

Behavioral procedures were identical to those of Experiment 1. However, in this experiment rats were given 4 trials on the first day and 8 trials on each of the next 3 days to a total of 28 trials.

ChAT Activity

One week after training (3 weeks after ICV injection with STREP), rats were decapitated and heads were immediately

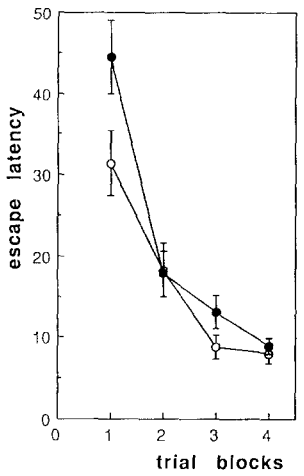

FIG. 1. Escape latencies (in seconds ± SEM) of 4-month-old control (○) and streptozotocin-treated (●) Lewis rats during acquisition of the Morris spatial navigation task.

immersed in liquid nitrogen for 8 s. Brains were taken out of the skull and the hippocampus was dissected at 0–4°C. Tissue samples were stored at −80°C until used to measure ChAT activity. Brain samples were homogenized (5%) in 50 mM phosphate buffer (pH 7.4) containing 0.2% Triton X-100 and 20 mM EDTA (pH 7.4). ChAT activity was determined according to the method of Fonnum (6), using [^{14}C]acetyl coenzyme-A (Amersham Corp., Arlington Heights, IL, 50–60 mCi/mM).

Statistical Analysis

Analysis of the Morris task and inhibitory avoidance data was identical to that of Experiment 1. Interdependencies of the different measures were analyzed using Pearson's correlation coefficient.

RESULTS AND CONCLUSIONS

STREP treatment did not affect the step-through latency during the first trial [step-through latency in seconds (SEM): CON, 29.8 (4.40); STREP, 27.0 (8.60); $t(13) = 0.29$, $p > 0.10$] and there was no treatment effect on inhibitory avoidance learning [difference score in seconds (SEM): CON, 229.4 (98.42); STREP, 158 (87.31); $t(13) = 0.53$, $p > 0.10$].

Analysis of the data for the acquisition of the Morris task revealed that rats reduced their escape latencies during training, $F(6, 78) = 14.07$, $p < 0.01$ (see Fig. 2) and that STREP-treated rats tended to have a poorer performance than control rats [general mean, $F(1, 13) = 3.74$, $0.10 > p > 0.05$].

Hippocampal ChAT activity (nM/mg protein/h) was decreased in STREP-treated rats [mean (SEM): CON, 83.6 (1.24); STREP, 45.0 (12.27); $t(13) = 9.03$, $p < 0.01$].

There was no correlation between inhibitory avoidance performance and hippocampal ChAT activity for either the CON or STREP group ($r = 0.20$ and -0.41, respectively; for both correlations, $p > 0.10$). However, hippocampal ChAT activity correlated negatively with the mean escape latency of the STREP group during the last four trial blocks of training in the Morris task, where the mean escape latency of the CON and STREP group diverged ($r = -0.86$, $p < 0.05$; see Fig. 3). No correlation between the two measures was found for the CON group ($r = -0.22$, n.s.). Low hippocampal ChAT activity thus predicted poor swimming performance in the Morris task in STREP-treated rats.

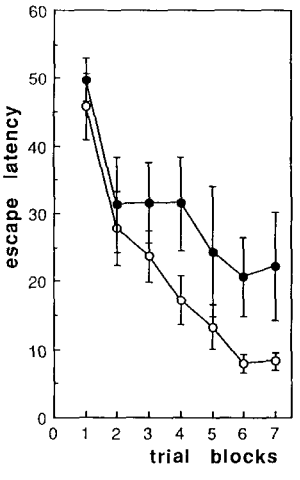

FIG. 2. Escape latencies (in seconds ± SEM) of 17-month-old control (○) and streptozotocin-treated (●) Lewis rats during acquisition of the Morris spatial navigation task.

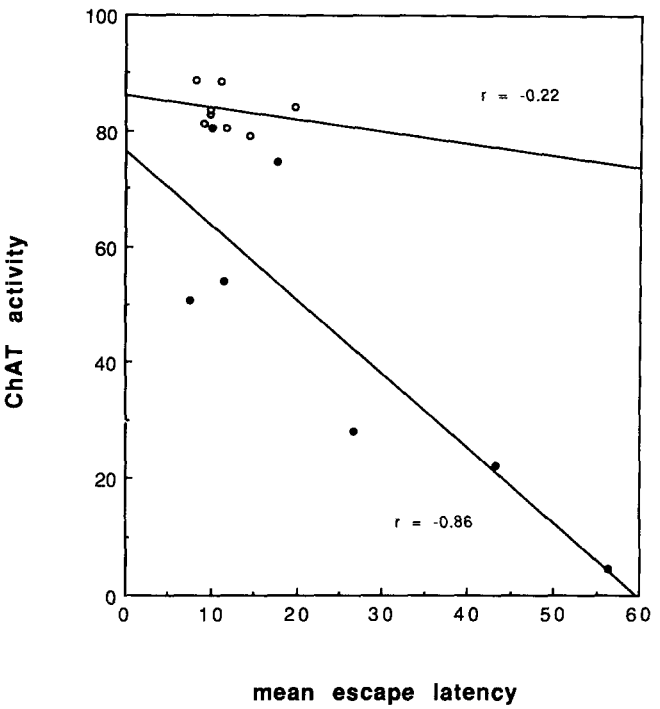

FIG. 3. Relation between hippocampal choline acetyltransferase activity (nM/mg protein/h) and average escape latency during the last four trial blocks of training in the Morris task for 17-month-old control (○) and streptozotocin-treated (●) Lewis rats.

GENERAL DISCUSSION

The present study showed that STREP treatment affected spatial discrimination performance in the Morris task at the end of training in middle-aged rats but not in young rats. This performance deficit of middle-aged STREP-treated rats was correlated with a decrease in hippocampal ChAT activity. Such a relation between spatial discrimination learning and the (hippocampal) cholinergic system has been found in other studies (3,9,18).

In contrast to the study of Mayer et al., in which 12-month-old Wistar rats were used (10), we did not observe a STREP-induced impairment in inhibitory avoidance learning in middle-aged rats. However, it should be mentioned that the mean difference score was higher for CON rats than for STREP rats but that the variation in both experimental groups was high. This large within-group variation may be due to the age of rats used in the present experiment (see also below). Another explanation for the different results could be that there were differences in the strain of rats used, apparatus, and parameters used in the inhibitory avoidance task.

An ICV injection of STREP in 3-month-old rats did not lead to a spatial discrimination performance deficit similar to that of rats treated with cholinergic antagonists. We assume that young rats are able to reverse/compensate for the effects of STREP treatment. However, young STREP-treated rats showed an impaired inhibitory avoidance performance,

thereby corroborating the results of Mayer et al. (10), who assumed that the shorter step-through latency during the test trial in the inhibitory avoidance task was not attributed to the STREP-induced increase in spontaneous locomotor activity because the step-through latency in the first trial did not differ for the STREP and control groups. It could, however, be argued that the STREP-induced increase in spontaneous locomotor activity might not be detected during the first trial because both the control and STREP groups had a short initial step-through latency. In the test trial, the STREP-induced increase in spontaneous locomotor activity could have decreased the step-through latency. These data should therefore be interpreted with caution. The absence of a STREP-induced learning impairment in the Morris task in young rats underscores the possibility that inhibitory avoidance learning in young STREP-treated rats is affected by an increase in spontaneous locomotor activity. Because inhibitory avoidance learning was not affected by STREP treatment in middle-aged rats, it is unlikely that the impaired spatial discrimination performance of these rats was affected by STREP-induced increase in spontaneous locomotor activity.

An ICV injection of STREP affects not only the cholinergic system but also the concentration of different monoaminergic neurotransmitters (noradrenaline, dopamine, and serotonin) in the rat brain differently (5). It could be argued that a change in these neurotransmitter systems could also have affected spatial discrimination performance in the Morris task. However, in the present study we found a strong correlation between escape performance and hippocampal ChAT activity, which suggests that there is a relation between the two measures. Such a correlation between cognitive function and ChAT activity has also been found in Alzheimer's disease (8,15). In addition, it has been found that, apart from the marked decrease in acetylcholine, Alzheimer's disease is accompanied by changes in different monoaminergic neurotransmitter systems [see (5)]. Thus, there is a resemblance in the decline in monoaminergic transmitter functions and a resemblance in relation between cholinergic function and cognitive processes in middle-aged STREP-treated rats and Alzheimer's disease. This observation suggests that the middle-aged STREP-treated rat can be regarded as an animal model for the cognitive decline observed in dementia.

Further, it has been suggested that the impairment of carbohydrate and related amino acid metabolism in Alzheimer's disease is related to an impairment of the insulin-insulin receptor system, also affected by ICV-administered STREP (13). A similar dysfunction of an underlying fundamental biochemical process may explain the similarities between the biochemical and cognitive changes in Alzheimer's disease and in the middle-aged STREP-treated rat. The present results encourage further investigation of the effects of STREP at both a behavioral and biochemical level.

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